available a complete procedure for the preparation of certain subcellular particulates from the crude tissue to the purified fractions without recourse to the centrifuge at any stage.

Some of the advantages of the short column technic for the preliminary preparation of cytoplasmic fractions are speed, simplicity of equipment and operation, an adaptability to a controlled gas phase during manipulation, and a sharp separation of cytoplasmic elements from nuclei and viable cells.

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On the Insulin Content of Pancreatic Juice.* (17588)

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It has been suggested that the islets of Langerhans are connected with and regenerated from duct tubules.(1) It is of interest to determine whether or not the internal secretions of the islands are present in the external pancreatic secretion. We have attempted to demonstrate insulin in fresh pancreatic juice by means of the "insulin effect" on rat diaphragm *in vitro*. The uptake of glucose by the isolated rat diaphragm has been extensively studied by Gemmill,(2) Stadie and Zapp,(3) Perlmutter and Greep,(4) Villee and Hastings, (5) and others. They have shown that glycogen synthesis by the rat diaphragm (measured directly or by glucose disappearance from the medium) is increased by the addition of insulin to the substrate.

Methods. Extraction. Pancreatic juice was extracted by modification of the insulin extraction procedures of Somogyi *et al.*(6) and Best and Scott.(7) Juice from the cannulated major pancreatic duct of the dog was collected into acid alcohol (95% ethyl alcohol plus 1.3% acetic acid) in proportion two parts of juice to three parts of acid alcohol. When 400 to 600 ml had been collected (usually over two to three days) the mixture was brought to pH 7.5-8.0 with several milliliters of 1 N NaOH and filtered. The filtrate was adjusted to pH 8.0 and concentrated *in vacuo* at 25-28°C for 24 to 48 hours. The residue was taken up in 50 to 100 cc of water, brought to pH 8.0 with .01 N NaOH, and filtered. To the filtrate was added ammonium sulphate 40 g/100 ml and the precipitate allowed to settle out overnight in the ice box. The precipitate was collected in a Buechner funnel on hard filter paper and then eluted repeatedly with 80% ethyl alcohol. The addition of an equal volume of ethyl ether caused a precipitate to appear. By repeated isoelectric precipitation was isolated that fraction soluble in water at pH 3.5 and 8, and insoluble at pH 5.0. This fraction was taken up in small amounts of water, buffered, and used for assay. To certain portions of pancreatic juice commercial insulin was added and the extraction carried out as above.

Assay. Well fed adult rats were killed by hammer and diaphragms quickly excised and placed in cold saline. Quarter diaphragms weighing 50 to 100 mg were placed in Warburg flasks. Each flask contained 2.0 ml of Krebs-Ringer phosphate solution buffered to pH 7.4, and containing 100 mg% of glucose, and 0.02

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TABLE I.

	Experiment	Glucose uptake mg/100 mg diaphragm/hr	% increase over control
1.	a. Diaphragm + substrate	.111	.0
	b. " + 0.01 μ insulin/ml	.248	124.3
	c. " + extr. equivalent to 7.1 ml of pancreatic juice/ml substrate	.136	22.5
	d. " + extr. of pancreatic juice to which insulin had been added .05 u./ml juice. Test extr. equivalent .9 ml juice/ml substrate	.231	108.1
2.	a. Same as 1a	.224	.0
	b. " " 1b	.484	116.1
	" " 1c	.284	26.8
	" " 1d	.364	62.5
3.	a. Diaphragm + substrate	.144	.0
	b. " + insulin 0.01 u./ml	.212	47.2
	c. " + extr. pancreatic juice to which insulin added .05 u./ml juice. Test extr. equivalent .93 ml juice/ml substrate	.289	100.7
	d. " + extr. 3c diluted 1/5. Test equivalent 0.18 ml juice/ml substrate	.234	62.5
	e. " + extr. 3c diluted 1/10. Test equivalent 0.09 ml juice/ml substrate	.238	65.3
4.	a. Diaphragm + substrate	.162	.0
	b. " + .01 u. insulin/ml	.406	150.3
	c. " + extr. pancreatic j. equivalent 8 ml juice/ml substrate	.200	23.1
	d. " + extr. pancreatic j. to which insulin added .022 u./ml. Test extr. equivalent 2.2 ml j./ml substrate	.370	128.4

ml of test substance. Two-tenths ml of 10% KOH was placed in center well, flasks gassed with oxygen, and shaken 80 times per minute at 38°C for 2 hours. At termination of incubation, aliquots of media were taken for glucose determinations by modifications of Folin-Wu(8) and Shaffer-Hartmann(9) methods.

Results. In preliminary experiments, concentrations of insulin of 0.005-0.01 unit per milliliter substrate regularly increased the utilization of glucose by diaphragm from 50 to 100%. The averages for 9 animals gave glucose utilization without insulin as .150 mg/100 mg diaphragm/hour, and with added insulin of 0.01 u./ml .285 mg/100 mg diaphragm/hour. These values are in good agreement with those reported by others. We could obtain no consistent effect at concentrations of 0.001 u./ml, nor did concentrations of insulin of 0.5 u./ml increase the effect. Results of experiments with extracts of pancreatic juice are summarized in Table I.

Summary. Commercial insulin added to fresh pancreatic juice can be extracted from the juice and its effect noted on the glucose uptake by the diaphragm in quantities as small as 0.0045 unit per milliliter juice.

In extracting pancreatic juice alone, no clear-cut demonstration of insulin effect (*i.e.*, an increase in glucose uptake of 50-100%) could be obtained, even though the extracts of as much as 7 milliliters of fresh juice per milliliter of substrate were tested. (The 20 to 25% averaged increase of glucose uptake by pancreatic juice is not of statistical significance.) Thus if insulin is present in pancreatic juice, the amount extractable and detectable by our methods must be less than .005/7 or .0007 unit per milliliter of juice.

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